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JOURNAL AND PROCEEDINGS  
OF THE  
ROYAL SOCIETY  
OF  
NEW SOUTH WALES

Volume 135 Parts 3 and 4  
(Nos 405-406)

2003

ISSN 0035-9173

PUBLISHED BY THE SOCIETY  
PO BOX 1525, MACQUARIE CENTRE, NSW 2113  
Issued April 2003

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ISSN 0035-9173

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## The 33rd Liversidge Lecture for the Royal Society of NSW

# Dietary Chemicals and Brain Function

GRAHAM A. R. JOHNSTON

### Abstract

Phytochemicals in our diet may play a vital role in maintaining the brain's chemical balance by influencing the function of receptors for the major inhibitory neurotransmitter GABA.

The flavonoids apigenin and epigallocatechin gallate, found in chamomile and green tea respectively, influence the way in which GABA receptors are modulated by drugs such as diazepam. Resveratrol, a flavonoid-like polyphenol found in red wine, acts on a subtype of GABA receptors consistent with its action as a cognitive enhancer. Bilobalide from *Ginkgo biloba*, a herb used in cognitive therapy, also influences GABA receptors.  $\alpha$ -Thujone, a terpenoid in the alcoholic beverage Absinthe, acts in a similar manner to bilobalide on GABA receptors. (+)-Borneol and other terpenoids from Valerian, a herb used to promote sleep, enhance the effects of GABA. The effects of these phytochemicals on GABA receptors are consistent with the overall actions of the beverages and herbal preparations that contain them, thus providing a rational basis for the use of these beverages and herbal preparations.

These studies provide evidence that chemicals in our diet may influence brain function in a positive way. The chemical nature of these substances may lead to the development of new therapeutic agents for the treatment of anxiety, epilepsy, memory disorders and insomnia.

**Keywords:** Brain function, chemicals, diet, balance, dosage

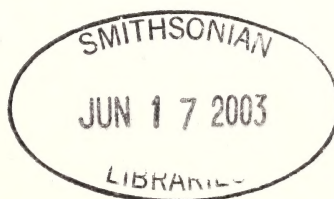
## THE BRAIN'S CHEMICAL BALANCE

Two simple chemicals, glutamic acid and GABA (Figure 1), are responsible for most of the communication between nerve cells in the brain. Indeed, at a very simple level, brain function may be thought of as a balance between excitation mediated by glutamic acid and inhibition mediated by GABA.

All nerve cells in the brain have receptors for glutamic acid and GABA. Some 40% of nerve cells release glutamic acid as an excitatory neurotransmitter, while a different 40% release GABA as an inhibitory neurotransmitter. The balance between these two chemical transmitters is vital to normal brain function. An excess of excitation over inhibition results in an

over excited brain (as in Figure 1) that can be manifested as anxiety, agitation, exhilaration, convulsions and death. On the other hand, an excess of inhibition over excitation can be manifested by depression, anaesthesia, coma and death. The particular manifestations of such imbalances in the brain depend on what neuronal circuitry is involved.

Ethanol is an example of a chemical that acts on both sides of the brain's chemical balance. The CNS depression that results from ingestion of ethanol is due to a reduction in excitation mediated by glutamic acid acting on a subtype of glutamate receptors known as NMDA receptors and to an enhancement of inhibition mediated by GABA acting on GABA<sub>A</sub> receptors.



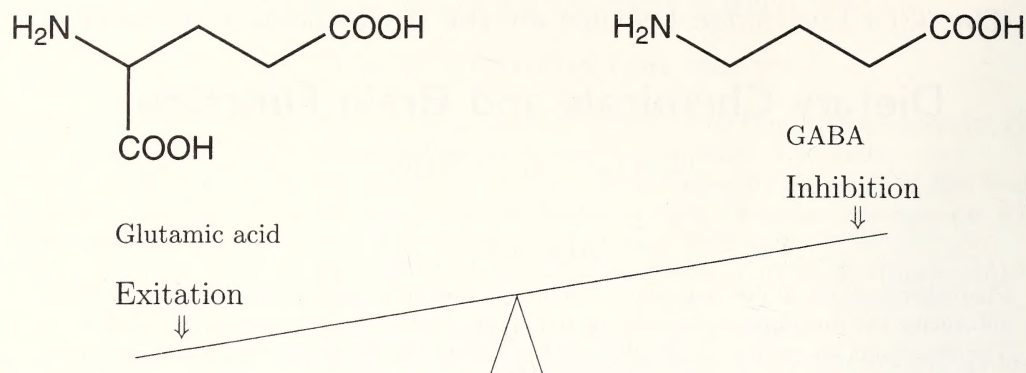


Figure 1: The brain's chemical balance between excitation mediated by the major excitatory neurotransmitter, glutamic acid, and inhibition mediated by the major inhibitory neurotransmitter, GABA.

## GABA RECEPTORS

GABA (whose name is derived from the old chemical name,  $\gamma$ -aminobutyric acid) acts on three main types of receptor to influence brain function. GABA<sub>A</sub> and GABA<sub>C</sub> receptors are fast acting receptors that belong to the group of receptors called ligand-gated ion channels (LGICs) (Chebib and Johnston, 2000). GABA acts as the ligand gating these receptors to open channels specific for chloride ions, allowing these ions to flow rapidly into nerve cells making them more negative and thus harder to excite. GABA<sub>B</sub> receptors act more slowly, inducing metabolic changes in nerve cells and belong to the group of receptors called G-protein coupled receptors (GPCRs) (Bowery et al. 1997).

The study of GABA receptors has been revolutionised by the introduction of recombinant receptor technology whereby receptors cloned from human brain are expressed in cells that do not normally express such receptors (Barnard et al. 1998). The recombinant receptors so formed may be studied in relative isolation using standard electrophysiological methodology. Since all GABA receptors are made up of protein subunits, recombinant receptors of known subunit composition may be studied.

The most common way to study recombinant GABA receptors is to express them in

oocytes from the South African frog, *Xenopus laevis* following injection of either DNA or RNA cloned from human brain and coding for particular GABA receptor protein subunits. These oocytes have the necessary cellular machinery to make the human proteins and assemble them on the surface of the oocytes as functional GABA receptors. The oocytes are approximately one millimetre in diameter and readily penetrated by glass microelectrodes. Using 2-electrode voltage clamp methodology, the effects of chemicals on the function of these GABA receptors may be assessed in a convenient quantitative manner. For example, using recombinant receptor technology, the effects of anti-anxiety agents such as diazepam (Valium) on GABA receptors can be easily shown to be restricted to a specific sub-group of GABA<sub>A</sub> receptors. The technology is not restricted to the study of pure chemicals – relatively crude mixtures of chemicals can be studied, for example to follow the purification of chemicals acting on GABA receptors from extracts of herbal products.

## FLAVONOIDS AND TERPENOIDS

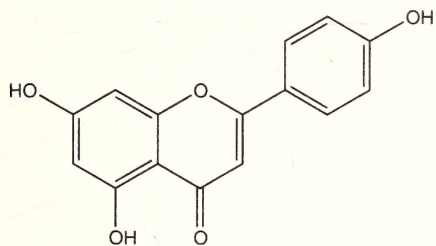
Flavonoids are polyphenolic chemicals widely distributed in the plant kingdom particularly in flowering plants. Flavonoids are responsible for many of the brilliant colours of fruits

and vegetables and are important constituents of red wine, green tea and many herbal preparations (Aherne and O'Brien, 2002). Chemically, flavonoids are C<sub>15</sub> compounds based on the chromane ring structure. Flavonoids have been studied extensively as anti-oxidants and oestrogens (Collins-Burow et al. 2000). Many of them show anti-cancer and anti-viral properties (Le Marchand, 2002).

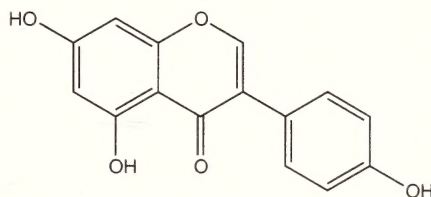
There is an extensive literature on the effects of flavonoids on GABA receptors (for a recent review see Marder and Paladini (2002), dating from the discovery of some plant derived isoflavans in bovine urine that inhibited the binding of diazepam to brain membranes (Luk et al. 1983). In the present context of actions on GABA receptors, the following flavonoids are of interest: the flavone apigenin; the isoflavone genistein; the flavanone naringenin; and the fla-

van, epigallocatechin gallate (Figure 2).

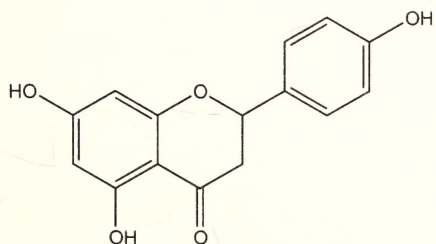
Terpenoids are also widespread in plants, especially in what are known as essential oils that can be extracted from plants and have a wide range of uses from perfume constituents to paint thinners. Terpenoids are oxygenated products formally derived from C<sub>5</sub> isoprene units and are classified by the number of C<sub>5</sub> units in their structure. Thus monoterpenoids have 2x C<sub>5</sub> units, sesquiterpenoids 3x C<sub>5</sub> units, diterpenoids 4x C<sub>5</sub> units and triterpenoids 6x C<sub>5</sub> units. In the present context of actions on GABA receptors, the following terpenoids are of interest:  $\alpha$ -thujone, (+)-borneol, bilobalide and picrotoxinin (Figure 3). Picrotoxinin is widely used experimentally as a non-competitive antagonist of GABA<sub>A</sub> and GABA<sub>C</sub> receptors, however, its convulsant action restricts its therapeutic use (Chebib and Johnston, 2000).



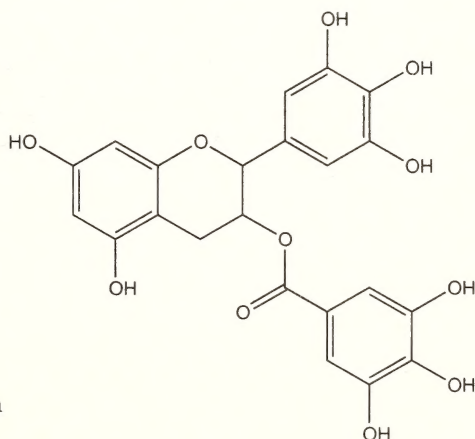
**Apigenin**, a flavone found in chamomile tea and related beverages.



**Genistein**, an isoflavone found in soy products, including tofu.

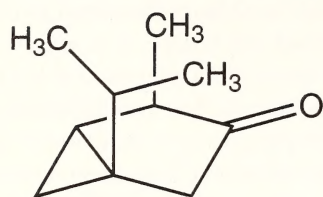


**Naringenin**, a flavone found in grapefruit.

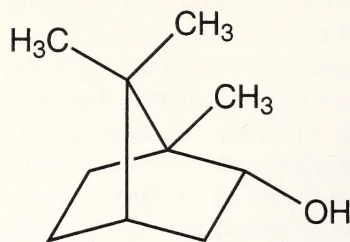


**(-)-Epigallocatechin Gallate**, a flavan found in green tea.

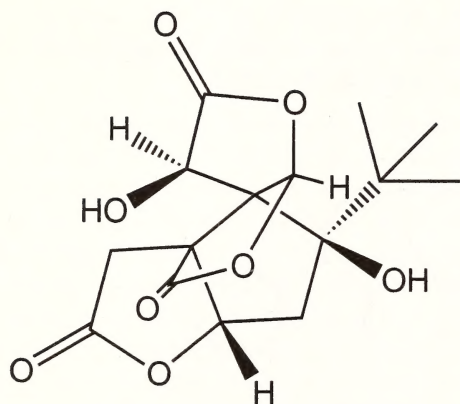
Figure 2: Some representative flavonoids



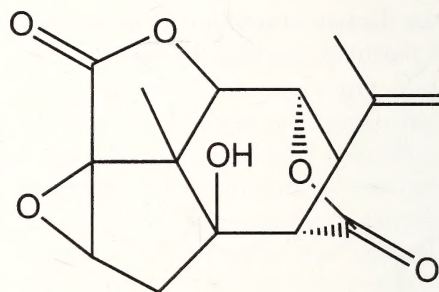
**$\alpha$ -Thujone**, a monoterpene  
from *Artemisia absinthium*



**(+)-Borneol**, a monoterpene  
from *Valerian officinalis*



**Bilobalide**, a sesquiterpenoid  
from *Ginkgo biloba*



**Picrotoxinin**, a sesquiterpenoid from  
*Anamirta cocculus*

Figure 3: Some terpenoids that influence GABA receptors

## APIGENIN FROM CHAMOMILE TEA

The lead compound for our investigations was apigenin (Figure 2), a flavonoid with a known anti-anxiety action found in chamomile tea. Chamomile tea is used widely to treat anxiety and insomnia. Current therapeutic drugs used for the treatment of anxiety and insomnia such as the benzodiazepines Valium and Serepax act at GABA<sub>A</sub> receptors in the brain, increasing chloride flow into neurones, resulting in decreased neural activity. There were divergent reports of the effects of apigenin on GABA<sub>A</sub> receptors. Viola et al. (1995) concluded that apigenin is a benzodiazepine agonist, like diazepam. However, Avallone et al. (2000) concluded that apigenin was a benzodiazepine inverse agonist (the exact opposite of diazepam).

Viola et al. (1995) based their conclusion that apigenin is a benzodiazepine agonist on the ability of apigenin to displace the binding of radiolabelled benzodiazepines from rat brain membranes, coupled with benzodiazepine-like effects in a rodent model of anxiety. However, binding studies do not reliably distinguish between agonists, antagonists and inverse agonists. Indeed, on the basis of similar binding studies, Dekermendjian et al. (1999) concluded that apigenin was a benzodiazepine antagonist (that is, it binds to the benzodiazepine site, blocking the binding of benzodiazepine agonists and inverse agonists, without having any effect on GABA responses itself). Avallone et al. (2000) used electrophysiological recordings from rat neurones. This allowed a more direct investigation of the activity of apigenin and showed that apigenin inhibited currents due to GABA, an effect which was blocked by the benzodiazepine antagonist, flumazenil. This fits the profile of a benzodiazepine inverse agonist. However, Avallone et al. (2000) did find some behavioural effects of apigenin which could be consistent with an action as a benzodiazepine agonist.

As part of her PhD studies, Erica Camp-

bell in our research group investigated the action of apigenin on recombinant GABA receptors. She used electrophysiological recordings from *Xenopus laevis* oocytes injected with recombinant human RNA for the most common subtype of GABA<sub>A</sub> receptor ( $\alpha_1\beta_2\gamma_2L$ ) in the brain. The actions of GABA at these receptors are enhanced by a variety of modulators including barbiturates, benzodiazepines, ethanol, and neuroactive steroids.

She showed the action of apigenin on the GABA<sub>A</sub> receptor is more complex than suggested by earlier studies. The effects of apigenin were biphasic dependent on the dose used. Moderate doses of apigenin inhibited the actions of GABA, diazepam and the steroid allopregnanalone, whereas low apigenin concentrations enhanced the effects of diazepam only (Figure 4). These effects are unlikely to be due to a simple action at the benzodiazepine site, suggesting a new site on the GABA<sub>A</sub> receptor.

While the inhibitory actions of apigenin at moderate doses were consistent with it acting as a benzodiazepine inverse agonist, the ability of apigenin to enhance the enhancing action of diazepam was novel. At low doses, apigenin had no direct effect on the action of GABA on these recombinant receptors. The presence of diazepam was necessary in order to observe the enhancing effects of apigenin. Thus apigenin appeared to be modulating the action of a modulator, an action not previously described in the pharmacological literature. Apigenin might be described as a second order modulator that influences the modulatory action of diazepam as a first order modulator on the activation of GABA<sub>A</sub> receptors.

The second order modulation of GABA<sub>A</sub> receptors by apigenin requires the presence of GABA and a first order modulator acting at a benzodiazepine site. The sedative and anxiolytic actions of apigenin observed in rodents (Avallone et al. 2000, Viola et al. 1995) can be interpreted on the basis of apigenin potentiating the action of endogenous benzodiazepine-like agents in the brain. Evidence for the physiologically relevant presence of such agents, termed

endozepines, has come from the discovery of a mutant GABA<sub>A</sub> receptor in childhood absence epilepsy and febrile seizures that has diminished sensitivity to benzodiazepines with little other alteration in GABA<sub>A</sub> receptor function (Wallace et al. 2001).

Genistein (Figure 2), an isoflavone found in soy products, did not show the biphasic effects of apigenin. Genistein, a structural isomer

of apigenin, showed only the GABA<sub>A</sub> antagonist action of apigenin. In addition, a dihydro derivative of apigenin, naringenin (Figure 2), a flavanone found in grapefruit juice and other citrus products, also showed only the GABA<sub>A</sub> antagonist action of apigenin. Thus, the second order modulatory action of apigenin is structurally specific.

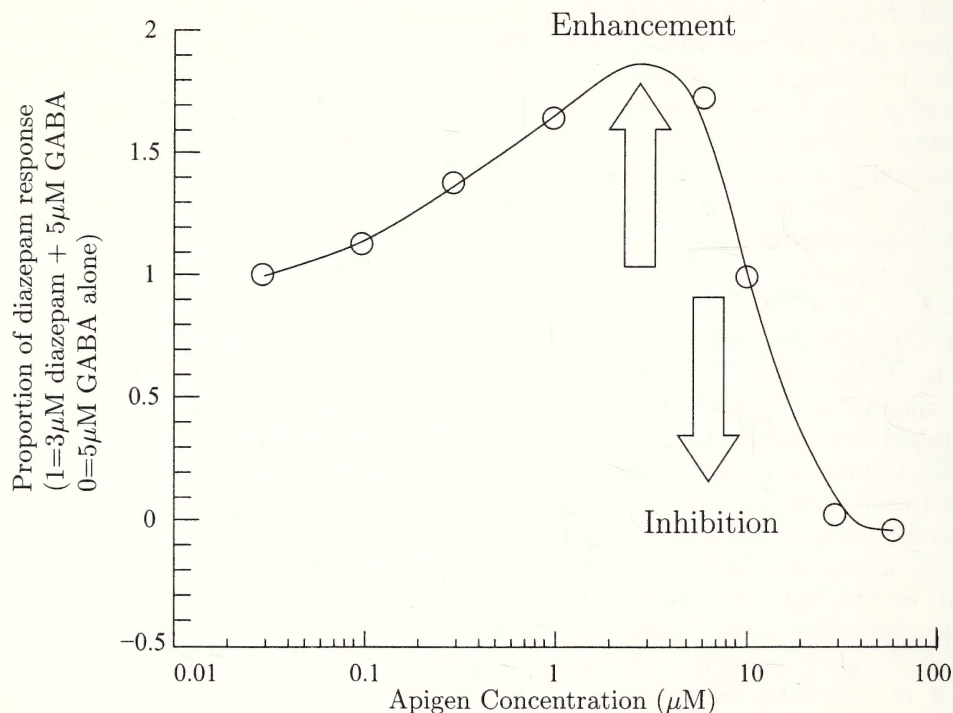


Figure 4: Effects of apigenin on the enhancement by diazepam of the action of GABA on recombinant GABA<sub>A</sub> receptors (Campbell et al. 1999).

## EPIGALLOCATECHIN GALLATE FROM GREEN TEA

Epigallocatechin gallate (Figure 2, EGCG) is the most abundant flavan in green tea (*Camellia sinensis*). It is found in all teas made from *C. sinensis* but not in many other food products (Arts et al. 2000b). Green tea is known to have many beneficial effects, including prevention of cancer, lowering of blood pressure and lipids, and acting as an antioxidant. EGCG has been

shown to contribute to these effects and, in addition, has been shown to have neuroprotective properties.

Erica Campbell investigated the actions of EGCG on recombinant GABA receptors (Campbell et al. 1999). She found that it shared the same biphasic action of apigenin, enhancing the action of diazepam at low concentrations and inhibiting at higher concentrations. In both the enhancement and inhibition phases, EGCG was at least 10 times more potent than apigenin.

(+)-Catechin and (-)-epicatechin, the most abundant flavans in nature, being found in many foods (Arts et al. 2000a, Arts et al. 2000b), did not influence recombinant GABA receptors, showing that the basic flavan ring structure is not sufficient for either of the actions of EGCG observed on recombinant GABA<sub>A</sub> receptors.

The biphasic actions of apigenin and EGCG emphasise the importance of dose in drug action. Our experiments show that low doses of apigenin and EGCG can enhance the activation of GABA receptors under the right conditions and thus could produce sedation and relief of anxiety. On the other hand, higher doses have the opposite effect and thus are likely to produce stimulation.

The second order modulatory action of apigenin and EGCG might have therapeutic possibilities. Low doses of these substances could reduce the therapeutic dose of diazepam and related benzodiazepines, while higher doses might reduce the effectiveness of such benzo-

diazepines. The possibilities of interactions between benzodiazepine medication and the consumption of chamomile and green tea need to be considered, particularly as chamomile tea may be used as a home remedy for those conditions for which benzodiazepines are frequently prescribed.

## RESVERATROL FROM RED WINE

The relatively low incidence of coronary heart diseases in France, despite intake of a high-fat diet, – the “French Paradox” – has been attributed to the consumption of red wine containing high levels of polyphenolic compounds (Mojzisova and Kuchta 2001, Sun et al. 2002). Resveratrol (3,4',5-trihydroxystilbene, Figure 5) is one of the most interesting polyphenolic compounds found in red wine. It has been shown to have estrogenic (Turner et al. 1999) and neuroprotective effects (Bastianetto et al. 2000).

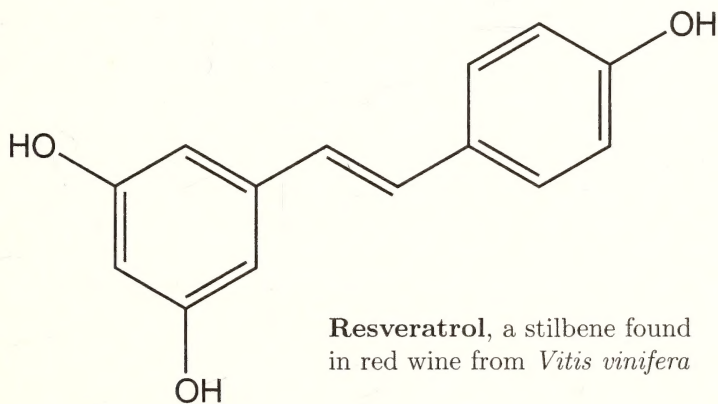


Figure 5: Structure of Resveratrol

In view of the structural similarities between resveratrol and apigenin, we investigated its effects on recombinant GABA receptors expressed in oocytes. To our surprise, resveratrol showed little action on GABA<sub>A</sub> receptors but was a GABA<sub>C</sub> receptor antagonist (Campbell and Johnston, 2003). Resveratrol non-

competitively inhibited the effects of GABA (1  $\mu$ M) at GABA<sub>C</sub> receptors with an IC<sub>50</sub> of 72  $\mu$ M, while having no significant effect at doses up to 100  $\mu$ M on the effects of GABA (40  $\mu$ M) at GABA<sub>A</sub> receptors. This is the first report of a non-competitive antagonist showing some selectivity for GABA<sub>C</sub> over GABA<sub>A</sub>

receptors, the widely used non-competitive antagonist picrotoxinin being some 30 times more potent at GABA<sub>A</sub> than at GABA<sub>C</sub> receptors (Chebib and Johnston, 2000).

We have a patent on the use of GABA<sub>C</sub> receptor antagonists to enhance cognitive activity and stimulate memory capacity (Johnston et al. 1998). Thus it was interesting to note that resveratrol has also been patented for the treatment of mild cognitive impairment (Wurtman and Lee, 2002) based on its ability to increase the expression of soluble amyloid precursor protein.

Using resveratrol as a lead compound, we are examining structural analogues to see if we can develop more potent and selective compounds acting on GABA<sub>C</sub> receptors. Recently we discovered a range of very promising activities, including the ability to enhance GABA<sub>C</sub> receptor activity, in a series of compounds synthesized in the 1970s by David Collins and colleagues in Veterinary Physiology at The University of Sydney as antiestrogenic and antifertility agents (Collins et al. 1971).

There is great interest in drugs to treat memory impairment in disorders such as Alzheimer's disease and schizophrenia. The use of such "Smart Drugs" in healthy people to increase their cognitive ability raises a variety of ethical, legal and social issues (Rose 2002).

Resveratrol and related stilbenoids are found in a variety of plants and herbs. Major dietary sources include grapes, wine, peanuts and soy (Burns et al. 2002). These compounds are also found in Itadori tea which has long been used in Japan and China as a traditional remedy for heart disease and stroke. For people who do not wish to consume alcohol, Itadori tea may be a substituent for red wine as a dietary source of resveratrol (Burns et al. 2002). For those who prefer white wine to red, French winemakers have created a Chardonnay, called Paradoxe Blanc, that is enriched in polyphenols and has been shown to be effective in reducing oxidative stress in diabetic rats (Landrault et al. 2003).

As noted above, ethanol itself enhances the

effectiveness of GABA acting on GABA<sub>A</sub> receptors and there is evidence that moderate consumption of alcoholic beverages is beneficial to health. However, other substances in these beverages, such as resveratrol, are likely to contribute to the overall beneficial effects. Recently it has been reported by Aoshima and colleagues (Hossain et al. 2002) that the fragrance of whiskey is able to enhance the effectiveness of GABA acting on GABA<sub>A</sub> receptors. Several components of the fragrance showed this property, the most potent being ethyl phenylpropionate, which was shown to have an anticonvulsant action in mice on inhalation.

Enhancement of GABA action was also found in extracts of other alcoholic drinks such as wine, sake, brandy and sochu. Hossain et al. (2002) noted "Although these fragrant components are present in alcoholic drinks at low concentrations (extremely small quantities compared with ethanol), they may also modulate the mood or consciousness of the human through the potentiation of the GABA<sub>A</sub> receptor response". Aoshima and colleagues have shown that various perfume constituents and aromatherapy agents potentiate GABA<sub>A</sub> receptors (Aoshima and Hamamoto 1999, Aoshima et al. 2001).

Clearly there are many interesting and innovative ways to explore the possibilities of influencing cognitive function.

## BILOBALIDE FROM GINKGO BILOBA

Extracts of *Ginkgo biloba* leaves are widely employed as herbal medicines to treat symptoms associated with mild-to-moderate dementia, impairment of other cognitive functions associated with ageing and senility and related neurosensory problems (Diamond et al. 2000). A study has indicated that the cognition-enhancing effects of the *Ginkgo* leaf extracts may be partly mediated by bilobalide via GABA receptors (Sasaki et al. 1999b). Enhanced hippocampal pyramidal neuronal excitability has been shown to correlate with learning and memory (Power

et al. 1997). Bilobalide (Figure 3), a sesquiterpenoid isolated from *Ginkgo biloba* leaves, has been shown to enhance this excitability in rat hippocampal slices, an action proposed to involve blockade of GABAergic neurotransmission (Sasaki et al. 1999b).

In collaboration with Sasaki and colleagues, Shelly Huang and Rujee Duke in our research group have shown that bilobalide is a potent antagonist of the action of GABA on recombinant GABA<sub>A</sub> and GABA<sub>C</sub> receptors (Huang et al. 2003). Bilobalide was only marginally less potent than picrotoxinin in these actions but there were subtle differences between the actions of bilobalide and picrotoxinin. These findings strongly support the proposal by Sasaki and colleagues (Sasaki et al. 1999b) that the observed enhanced neuronal excitability in hippocampal slices was due to its blockade of GABAergic neurotransmission.

Bilobalide and picrotoxinin share common structural features, including a hydrophilic cage and lipophilic side chain. However, bilobalide and picrotoxinin have opposite actions upon systemic administration to animals. Bilobalide is an anticonvulsant (Sasaki et al. 1999a,b) whereas picrotoxinin is a potent convulsant (Jarboe et al. 1968). There are, however, only minor differences in their activities at recombinant GABA<sub>A</sub> and GABA<sub>C</sub> receptors. Bilobalide has been shown to increase GABA levels in the hippocampus and cerebral cortex of mice (Sasaki et al. 1999a). This increase may override the GABA<sub>A</sub> antagonist action of bilobalide that would be expected to produce convulsions and result in the overall anticonvulsant action. Nonetheless, the induction of epileptic seizures by *Ginkgo* extracts has been noted in rare cases (Granger, 2001). The anticonvulsant/convulsant actions of bilobalide need further investigation and may provide vital clues as to the safe use of *Ginkgo* extracts in the treatment of mild cognitive deficits.

*Ginkgo* leaves were used traditionally in Japan to protect books against harmful worms and insects before the introduction of modern insecticides. Like picrotoxinin, bilobalide is a

potent insecticide (Ahn et al. 1997), an action likely to be due to blockade of insect GABA receptors.

## THUJONE FROM ABSINTHE

Absinthe was the favoured drink of artists and writers in Paris at the end of the 19th century. The emerald green liqueur made famous by Van Gogh, Toulouse-Lautrec and their colleagues was banned in France and most other countries by 1915 due to its ability to cause convulsions, hallucinations and psychotic disturbances.

The toxic component of absinthe has been identified as the monoterpenoid  $\alpha$ -thujone (Figure 3). It is also the active ingredient of wormwood oil and some other herbal medicines and is reported to have antinociceptive, insecticidal, and anthelmintic activity. Hold et al. (2000) showed that  $\alpha$ -thujone acted like picrotoxinin as a GABA<sub>A</sub> receptor non-competitive antagonist. They showed that  $\alpha$ -thujone was rapidly metabolised to less active metabolites and concluded that " $\alpha$ -thujone in absinthe and herbal medicines is a rapid-acting and readily detoxified modulator of the GABA-gated chloride channel".

Matthew Roper in our research group has shown that  $\alpha$ -thujone is a non-competitive inhibitor of the action of GABA on recombinant  $\alpha_1\beta_2\gamma_2L$  GABA<sub>A</sub> and  $\rho_1$  GABA<sub>C</sub> receptors expressed in oocytes. Like picrotoxinin,  $\alpha$ -thujone was about 30 times more potent at GABA<sub>A</sub> than at GABA<sub>C</sub> receptors. Furthermore, site-directed mutagenesis studies showed that mutations in the second membrane-spanning region of the wildtype GABA<sub>C</sub> receptors influenced the potency of  $\alpha$ -thujone and picrotoxinin in a similar manner indicating that both convulsants interact with the same amino acid residues on the GABA<sub>C</sub> receptor.

Many plant-derived essential oils, such as wormwood, have been known for over a century to have convulsant properties. Burkhard et al. (1999) reported on case studies of plant-related toxic seizures related to use of these oils

for therapeutic purposes. They noted that “the literature shows essential oils of 11 plants to be powerful convulsants (eucalyptus, fennel, hysop, pennyroyal, rosemary, sage, savin, tansy, thuja, turpentine, and wormwood) due to their content of highly reactive monoterpene ketones, such as camphor, pinocamphone, thujone, cineole, pulegone, sabinyl acetate, and fenchone.” They went on to state “Nowadays the wide use of these compounds in certain unconventional medicines makes this severe complication again possible”.

Absinthe is now available in a less potent form that contains less than 10 parts per million of  $\alpha$ -thujone, whereas traditional absinthe contained more than 250 parts per million.

## BORNEOL FROM VALERIAN

Valerian (*Valeriana officinalis*) is a medicinal plant used widely throughout the world. Extracts of the dried underground parts of the plant are used to relieve anxiety, restlessness and nervous sleep disorders. There is evidence of its use by the ancient Greeks, Romans and Chinese for healing purposes. Early herbalists and physicians such as Hippocrates, Galen and Culpeper noted the sedative and digestive properties of valerian, advocating its use as a muscle relaxant, diuretic, expectorant and wound healer (Plushner, 2000). Today there are over 400 commercially available products containing valerian in Germany, more than 80 in the United Kingdom and more than 30 available in Australia (Houghton, 1999; Shohet et al. 2001).

Valerian extracts may be considered to be a “herbal Valium”, given that they have a benzodiazepine-like action reducing the latency of sleep onset and increasing the depth of sleep and the perception of well-being. These extracts contain a large number of constituents, many of which are thought to be active at GABA receptors. Compounds that have been identified include acids (valerenic acid and isovalerenic acid), ketones (valeranone), alcohols (valerenol, maaliol), aldehydes (valerenal) and valepotriates (valtrate, isovaltrate). Valerian extracts

also contain various flavonoids, alkaloids, tannins and amino acids.

Renee Granger in our research group obtained 2 kg of the dried underground parts of *Valeriana officinalis* from Newton's Herbal Pharmacy in Sydney. She extracted this with hexane, ethyl acetate, methanol and methanol:water (1:5), and fractionated the extracts using silica gel chromatography. This procedure produced more than 450 fractions, which were assessed using thin layer chromatography and functional studies on recombinant receptors many fractions influenced GABA action on GABA<sub>A</sub> and GABA<sub>C</sub> receptors.

Dried valerian root normally contains 0.3–0.8% volatile oil, including borneol, valerenic acid, valeranone and kessyl glycol. These essential oil fractions proved very difficult to purify, so pure compounds were purchased and tested on recombinant receptors. This produced a very surprising result.

(+)-Borneol, the natural enantiomer found in Valerian, produced a 12 fold enhancement of the action of 10  $\mu$ M GABA on recombinant GABA<sub>A</sub> receptors under conditions whereby diazepam would give at best a 2 fold enhancement (Figure 6; Granger et al. 2002). While relatively high concentrations of (+)-borneol were needed (EC<sub>50</sub> 400  $\mu$ M), this degree of enhancement is unprecedented.

Under these conditions, (-)-borneol produced a 4 fold enhancement (EC<sub>50</sub> 450  $\mu$ M), isoborneol a 7 fold enhancement (EC<sub>50</sub> 680  $\mu$ M), while the structurally related monoterpenes camphor, bornyl acetate and *p*-cymene each produced an approximately 3x potentiation (with EC<sub>50</sub>'s around 300  $\mu$ M).

While many general anaesthetics, barbiturates and benzodiazepine are known to produce up to 2 fold enhancement of the response of GABA<sub>A</sub> receptors to GABA (Belelli et al. 1999b), the general anaesthetic etomidate is known to cause much larger enhancements, particularly at mutated GABA receptors, e.g. etomidate produces a 10 fold enhancement of GABA responses at GABA<sub>C</sub> receptors where the wild type isoleucine residue at position 307

had been mutated to a serine (Belelli et al. 1999a).

(+)-Borneol represents an intriguing structural lead for the development of a new class of therapeutic agents acting on GABA receptors. Purified (+)-borneol has been used for medicinal purposes in China and Japan (Hattori, 2000).

Borneol is a common constituent of the essential oil component of many plants and thus a component of many herbal preparations. On the basis of our studies, (+)-borneol would be expected to have antianxiety, anticonvulsant and sedative properties.

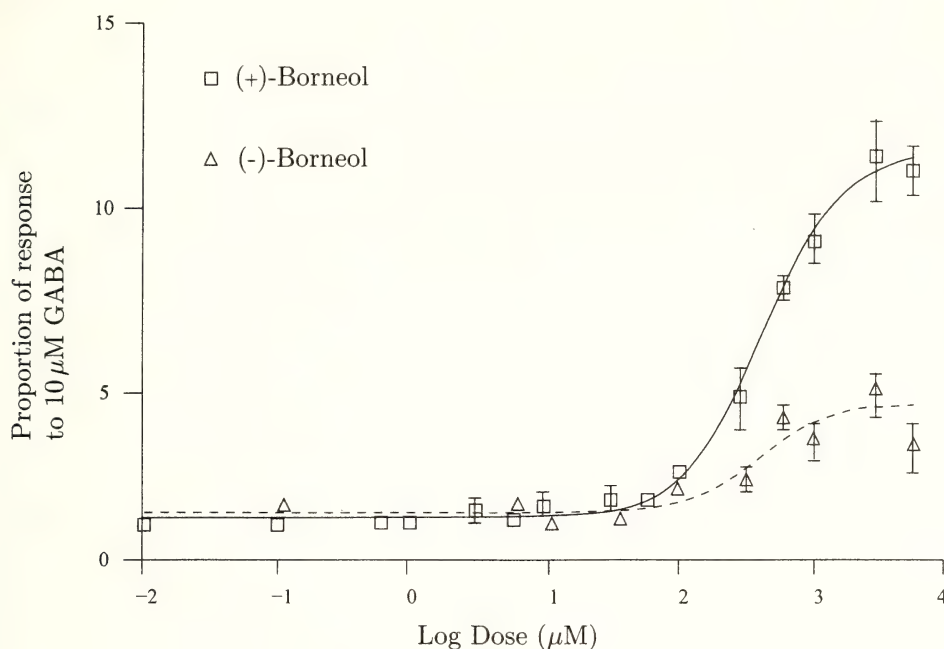


Figure 6: Dose-response curve for the potentiation of the response to  $10\mu\text{M}$  GABA by (+)- and (-)-borneol at recombinant  $\text{GABA}_A$  receptors (Granger et al. 2002).

## NATURAL VERSUS SYNTHETIC

Natural products derived from plants provided the first medicines. These were complex mixtures of chemicals. The active principles in these mixtures were isolated and developed as single chemical entities to use as drugs and from which to develop new therapeutic agents. The development of aspirin from the salicylates found in Willow bark is a classic example of this. Natural products continue to be an important source of new drugs. There are sophisticated laboratories in many countries, including

Australia, using high throughput technology to screen extracts of natural products for desired biological activities.

Herbal preparations are by their nature mixtures of chemicals. It is a basic tenet of herbal medicines that the whole is more than the sum of the parts. The various chemicals in herbal preparations are considered to act together in a synergistic way to effect treatment of particular disorders. This is in direct contrast to the “magic bullet” approach of single chemical entity drugs designed to hit a particular target in a highly selective manner. Both approaches have

their role in promoting health and well-being.

There is a widespread belief on the part of the general public that natural substances are inherently superior to synthetic substances with regard to efficacy and safety in matters related to human health. This question has been addressed recently by a task force of the International Union of Pure and Applied Chemistry (Topliss et al. 2002). A comparison of the characteristics of natural and synthetic substances used in a variety of therapeutic drugs, herbal medicines, vitamins and nutrients shows a similar range of favorable and unfavorable effects. It was apparent that molecular structure and dose determine the effect of chemicals on human health, not whether they are of natural or synthetic origin.

Natural chemicals such as many flavonoids have been consumed in our diet for countless generations. This suggests that they would be unlikely to have serious adverse effects severe enough to prevent their use as therapeutic agents. However, it is likely that the overall balance of flavonoids and related natural chemicals in our diet is of vital importance, given the examples in this review of such chemicals having opposing actions on GABA receptors. Recombinant receptor technology offers the means to assess the overall effects of complex mixtures of chemicals on the functioning of key receptors. Such technology is expected to play an increasingly important role in the quality control of herbal preparations and "functional foods".

Herbal preparations can have significant interactions with therapeutic drugs, for example by altering the metabolism of these drugs and thus influencing their potency and duration of action (Izzo and Ernst 2001). It is important that health care professionals ask their patients about their use of herbal products and consider the possibility of herb-drug interactions. Food-drug interactions are also important (Sorensen 2002) as many naturally occurring substances influence the cytochrome p450 enzymes that play such an important role in drug metabolism. Grapefruit juice is a classic example. It contains substances, including the flavonoid narin-

genin (Figure 2), that inhibit the p450 enzyme CYP3A4 resulting in higher bioavailability of drugs with a high firstpass metabolism (Fuhr 1998). While there may be a place for grapefruit juice as a drug-sparing agent, more research is needed and drugs possibly influenced by the consumption of grapefruit juice should be appropriately labelled.

## CONCLUSIONS

GABA receptors in our brain are susceptible to a wide variety of chemicals consumed in the diet. Our GABA receptors exist in a milieu of substances that influences their function, often in opposing ways. The balance between the effects of these substances will determine at any particular point in time how the receptors respond to GABA, their natural neurotransmitter. This review has summarised the effects of some substances found in four beverages (chamomile and green tea, red wine, absinthe) and two herbal preparations (*Gingko* and *Valerian*) that have significant actions on recombinant GABA receptors consistent with the overall actions of the beverages and herbal preparations. The chemical nature of these substances may lead to the development of new therapeutic agents for the treatment of anxiety, epilepsy, memory disorders, and insomnia. Does the concept of dietary substances influencing brain function indicate that we have entered an era of neuraceuticals?

These studies provide a chemical basis for some of the effects that these beverages and herbal preparations have on brain function, and may lead to rational improvements in their quality control and use, especially in combination with other agents known to influence GABA receptors, such as alcohol, anaesthetics and benzodiazepines.

## ACKNOWLEDGEMENTS

It is a pleasure to gratefully acknowledge the collaboration in these studies of Erica Campbell, Mary Chebib, Rujee Duke, Renee Granger,

Belinda Hall, Jane Hanrahan, Shelly Huang, Ken Mewett, Matthew Roper and Hue Tran. Appreciation is also due to Gary Cutting, George Uhl and Paul Whiting for the supply of cloned GABA receptors and to the Australian National Health and Medical Research Council for financial support.

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- This paper is based on the 33<sup>rd</sup> Liversidge Lecture presented before the Royal Society of New South Wales at the Frencham School, Mittagong, 22nd November 2002.
- (Manuscript received 29.1.2003)



# Ionic Combating Mechanisms and their Comparative Effects on Seed Hardening under Simulated Supra-Optimal Environmental Conditions

M. A. KADER

## Abstract

Heat extremes and limited moisture are two of the most dominant environmental factors impacting stand establishment of rainfed sorghum (*Sorghum bicolor* L. Moench). Hardening is the process of exposing plants to gradual levels of stress and acclimation to foster better response to post-treatment stress factors. Three experiments were carried out under phytotron and germination cabinet conditions to test the effects of osmotic soaking of sorghum seeds with sodium chloride (NaCl) on germination and growth under simulated heat stress. The hypothesis was that the NaCl treatment forms an acclimation to stress by inducing hormonal responses to ionic toxicity caused by salt. This acclimation would lead to a lowered degree of response when the seed is exposed to future stress; namely, heat and/or drought. Independent variables included NaCl concentration, treatment duration, storage and genotype, whereas germination and growth were dependent variables. Further experiments tested various methods of achieving the initial acclimation “signaling” whilst reducing ionic toxicity through combating ions. Longer soaking treatment durations (2–3 days) and higher NaCl concentrations (16 g NaCl l<sup>-1</sup>) were detrimental to germination in comparison to lower concentrations (8 g NaCl l<sup>-1</sup>) and shorter durations (1 day). An interaction between concentration and duration of treatment existed where high concentrations performed better at lower treatment durations and vice versa. Combining 10 g NaCl l<sup>-1</sup> with 5 g calcium sulphate l<sup>-1</sup> to combat ionic toxicity produced a greater advancement of germination than NaCl alone. Drying duration of seeds did not affect subsequent germination nor did storage for 10 days to 1 month. The effects of osmotic conditioning are discussed and could have potential for improving sorghum success rates in harsh arid environments.

**Keywords:** ions, acclimation, stress, sorghum, germination

## INTRODUCTION

The fate of sorghum (*Sorghum bicolor* L. Moench) seeds sown into dry or gradually drying seedbeds that hamper emergence is not well known. Even though some seeds may germinate and give rise to seedlings, the majority will fail to emerge (Al-Mudaris and Jutzi 1998). This clearly sets back stand establishment and subsequent yield due to thermo and other forms of dormancy (Silvertown 1999). Treating seeds with osmotic solutions before sowing, also termed “osmoconditioning”, has been shown to improve germination and seedling emergence in a range of species (Heydecker and Gibbins 1978, Brocklehurst and Dearman 1984). The use of

sodium chloride (NaCl) as the osmotic agent in such treatments has also been investigated and shown to yield enhanced germination patterns in sorghum (Al-Mudaris 1998). Both the concentration of NaCl and treatment duration seem to play major roles in the response exhibited by seeds. However, little is known of the role of both factors or of the effect of various salt mixtures on germination and early seedling growth of treated seeds. This is especially the case when ionic toxicity is taken into account as this is the single most important factor affecting NaCl usage as a priming agent. Ionic combating by way of adding extra elements to the NaCl solution may aid in reducing the neg-

ative effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ions (Al-Mudaris and Jutzi 1999).

Conditioning whole plants to stress by gradual exposure to limited and increasing levels of the particular stress factor has been found to alleviate part of the stress at later stages due to an early peaking of abscisic acid (ABA) production and a better hormonal balance in favour of Kinetin (Al-Mudaris 1998a). The concept of conditioning seeds to heat stress by exposing them to pre-germination salinity stress may aid in "hardening" seeds and improving subsequent germination and seedling growth under heat stress.

This paper investigates the effects of NaCl concentration, treatment duration, salt mixtures and storage on germination and growth parameters in four sorghum genotypes. It also evaluates the effect of combining NaCl with other salts in advancing the "hardening" effect to improve germinative performance under drought and/or heat stress by way of ionic combating.

## MATERIALS AND METHODS

### Effect of Salt Concentration, Osmoconditioning Treatment Duration and Drying

Seed lots of the sorghum variety IRAT 204 were used in this set of experiments. Seed tests revealed a 1000 seed weight of 34 g, a mois-

ture content of 15%, viability of 100% and germinability of 98.0% following International Seed Testing Association (ISTA) rules (ISTA 1993).

Previous work (Al-Mudaris and Jutzi 1998 and 1998a) showed the upper range of positive responses to NaCl to lie between 10 and 20 g NaCl  $\text{l}^{-1}$ . Therefore, two concentrations around this range were used. These were 8 and 16 g NaCl  $\text{l}^{-1}$ . A water-soaked (distilled water) wet control was also evaluated. Sodium chloride solutions were prepared at the concentrations mentioned, and seeds soaked in them at 10°C in the dark inside glass beakers for one of three durations; namely 1, 2 or 3 days (d). The wet control was also soaked in water for the same periods of time. The 10°C incubation temperature proved low enough to prevent premature germination (visible signs of radicle emergence after imbibition) during soaking in water.

After treatment, seeds were either sown fresh without drying or surface dried by exposing to an airflow of 25°C for 3 hours (h) inside an incubator and sown dry. Seeds were sown in batches of 100 in 1000 mL polystyrene trays filled with equal volumes of sieved sand (weight basis) and irrigated to weight with 200 mL of water, initially, and as they lost one third of their weight thereafter. Trays were weighed daily at 7:00am. Experiments were conducted in a 18 m<sup>3</sup> walk-in phytotron (Heraeus-Voetsch, Germany) set at the environmental conditions shown in Table 1. These conditions were an attempt to simulate likely heat stress during the course of a day (Al-Mudaris 1998a).

Time (hh.mm)	Temperature (°C)	Relative Humidity (%)	Light (33 klux)
24.00 – 05.00	15	65	Absent
06.00 – 08.00	17	62	Activated
09.00 – 12.00	29	50	Activated
13.00 – 19.00	43	38	Activated
20.00 – 21.00	25	55	Activated
22.00 – 23.00	23	58	Absent

Table 1. The course of temperature, relative humidity and light activation in the phytotron during a 24 hour cycle.

Treatment combinations were replicated four times each at 100 seeds per replicate (3 treatments  $\times$  3 durations  $\times$  2 drying treatments  $\times$  4 replicates = 72 experimental units). Trays were arranged in a Randomized Complete Block Design (RCBD). Emergence counts were taken daily for 12 d and from them the final germination percentage (FGP), mean germination time (MGT) and germination index (GI) calculated. MGT and GI were calculated following Orchard (1977) and Benech Arnold et al. (1991), respectively. The MGT is a measure of the mean time taken for a seed lot to germinate, while the GI assigns maximum weight to a higher number of seeds germinating earlier (Al-Mudaris 1998). Data were arcsin transformed (Yang et al. 1999 and Houle et al. 2001) and analysed in ANOVA using the General Linear Model (GLM PROC) of the SAS<sup>®</sup> statistical package for Windows<sup>®</sup>.

At 12 days of age seedlings were collectively harvested from each tray, separated into shoots and roots, and, after washing roots under running tap water, dried at 80°C for 4 d in a reverse cycle oven (Convion Industries, Canada). Average dry weights of shoots (DWS) and roots (DWR) and the shoot:root ratio (SRR) were obtained and averaged. Also, after harvest, the contents of each tray were emptied into a sieve with 2 mm openings, and germinated but unemerged seeds retrieved and counted (hereafter termed GUE). Those that had neither germinated nor emerged were classified as non-germinated seeds and represented the difference between emerged and germinated unemerged seeds (Munir et al. 2001). These were subjected to a tetrazolium test of viability following ISTA (ISTA 1993) to verify their viability status, but data was not analysed. Individual seeds were also studied under a dissecting microscope for further viability and anatomy notes following Hidayati et al. (2001). Germination and growth data were exposed to one-way and two-way analysis of variance (ANOVA) procedures for single factors and interactive factors of seed treatment  $\times$  duration (Weber and D'Antonio 1999).

### Effect of Salt Concentration, Osmoconditioning Treatment Duration and Storage

The same NaCl treatments mentioned above (0, 8 and 16 g NaCl l<sup>-1</sup>) were used in combination with the same treatment durations (1, 2 or 3 d). After treatment, IRAT 204 seeds were dried back at 25°C for 24 h and stored at 22°C and 50 to 52% relative humidity for 30 d in the dark. Thereafter, seeds were retrieved from storage and sown in batches of 100 in 1000 mL polystyrene trays lined with creased filter paper. Each tray was moistened with 40 mL of a polyethylene glycol solution (PEG) (Fluka Chemie, Germany) and covered with a lid to minimise evaporation. The solution had an osmotic potential ( $\psi_s$ ) of -10 bar (circa -1.0 MPa) simulating drought (Marschner 1995, Dodd and Donovan 1999). The PEG molecular weight (m.w.) was 10,000. Trays were placed in an incubator set at 42/25°C (day/night, 12 h/12 h). Germination counts were taken daily for 12 d and from them the FGP, MGT and GI calculated. Statistical arrangements were similar to the first experiment with experimental factors being treatment and soaking duration applied in RCBD (Dodd and Donovan, 1999).

### Effect of Salt-Based Mixtures and Drying Duration

Three sorghum varieties were tested in this trial. These were ICSV 112, ICSV 745 and M35-1. Seed lot tests revealed germinability, moisture content and viability levels comparable to those of IRAT 204 sorghum used in the previous two experiments.

The hypothesis to be tested here was that mixing NaCl with other minerals might provide both the stress acclimation (hardening) effect and a mineral uptake effect, thus improving seed performance under stress. The "softening" effect of other elements in the mixture might, thus, lead to a balance and a combating of Na<sup>+</sup> and Cl<sup>-</sup> ions. A basic 10 g NaCl l<sup>-1</sup> ( $\psi_s$  of -7.7 bar) (Knauer Osmometer, Germany) treat-

ment was mixed with calcium sulphate or one of three fertilizers as follows:

- T<sub>1</sub>: Dry Control (dry, untreated seeds)  
 T<sub>2</sub>: 10 g NaCl l<sup>-1</sup> + 5 g CaSO<sub>4</sub>.2H<sub>2</sub>O l<sup>-1</sup>  
 (calcium sulphate)  
 T<sub>3</sub>: 10 g NaCl l<sup>-1</sup> + 7.5 g Urea fertilizer l<sup>-1</sup>  
 (water-soluble nitrogen-source fertilizer)  
 T<sub>4</sub>: 10 g NaCl l<sup>-1</sup> + 10 g NPK fertilizer l<sup>-1</sup>  
 (slow release compound fertilizer with  
 6% N, 12% P<sub>2</sub>O<sub>5</sub> and 18% K<sub>2</sub>O)  
 T<sub>5</sub>: 10 g NaCl l<sup>-1</sup> + 15 g DAP fertilizer l<sup>-1</sup>  
 (di-ammonium phosphate,  
 soluble phosphorous source)  
 T<sub>6</sub>: 10 g NaCl l<sup>-1</sup>

Seeds were soaked in the above mentioned solutions for 3 d at 18°C in the dark. The concentrations were based on earlier work with sorghum (Al-Mudaris 1998). After treatment, seeds were dried back at 25°C for 7, 14 or 21 h and stored for 1 month at 24°C in the dark. Subsequently, seeds were sown in batches of 100 in polystyrene trays at 40/20°C (day/night temperatures, 12h/12h) under a PEG-induced (m.w. 10,000) drought of -3.3 bar (0.3 MPa). The change in temperature down to 40/20°C was based on observation of partial fungal infection at the 42/25°C level in the previous trial. Again, the FGP and MGT were calculated in addition to the coefficient of velocity of germination (CVG) (Jones and Sanders 1987). The CVG increases when the number of germinated

seed increases and the time required for germination decreases. Theoretically, the highest CVG possible is 100 and would occur if all seeds germinated on the first day (Jones and Sanders 1987). Statistical procedures were the same as those for the second experiment.

## RESULTS AND DISCUSSION

### Effect of Salt Concentration, Osmoconditioning Treatment Duration and Drying

The 16 g NaCl l<sup>-1</sup> treatment reduced the FGP and GI in comparison to the wet control and the 8 g NaCl l<sup>-1</sup> treatment. Both salt soaks reduced the time needed to germinate as seen from lower MGT values (Table 2). Neither growth parameters (DWS, DWR, and SRR) nor the number of GUE seeds differed between treatments. The longer the duration of soaking in NaCl solutions, the lower the final germination percentage and germination speed, and the higher the number of GUE seeds (Table 3). Drying seeds, on the other hand, did not affect germination characteristics (FGP, MGT and GI) but reduced the DWR and, thus, increased the SRR (Table 4).

The interactive effects of NaCl concentration, soaking duration and drying on germination and growth characteristics of IRAT 204 revealed no significant interactions between treatment combinations regarding the parameters studied (data not shown)

Seed Treatment	FGP (%)	MGT (day)	GI	DWS (mg)	DWR (mg)	SRR	GUE (seeds)
Wet Control	78.8 a	4.3 a	278.6 a	6.6 a	9.4 a	0.75 a	4.7 a
8g NaCl l <sup>-1</sup>	73.2 a	3.5 b	262.2 a	6.6 a	9.1 a	0.75 a	4.2 a
16g NaCl l <sup>-1</sup>	62.1 a	3.5 b	228.0 b	6.3 a	8.6 a	0.84 a	6.0 a

Table 2. Effect of NaCl treatments on germination and growth characteristics of sorghum IRAT 204 under phytotron conditions.

Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time, GI: Germination Index, DWS: Dry Weight of Shoot, DWR: Dry Weight of Root, SRR: Shoot : Root Ratio and GUE: Germinated Unemerged Seeds.

Treatment Duration (days)	FGP (%)	MGT (day)	GI	DWS (mg)	DWR (mg)	SRR	GUE (seeds)
1	79.4 a	3.5 b	296.8 a	6.7 a	8.6 a	0.84 a	3.3 b
2	70.0 b	4.0 a	242.5 b	6.3 a	9.8 a	0.69 a	5.6 a
3	64.7 b	3.7 ab	229.5 b	6.5 a	8.7 a	0.80 a	5.9 a

Table 3. Effect of duration of soaking in NaCl solutions on germination and growth characteristics of sorghum IRAT 204 under phytotron conditions.

Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time, GI: Germination Index, DWS: Dry Weight of Shoot, DWR: Dry Weight of Root, SRR: Shoot : Root Ratio and GUE: Germinated Unemerged Seeds.

Drying Treatment	FGP (%)	MGT (day)	GI	DWS (mg)	DWR (mg)	SRR	GUE (seeds)
No Drying (Fresh Sown)	72.7 a	3.6 a	263.0 a	6.5 a	10.0 a	0.69 b	4.6 a
Drying (25°C, 3 h)	70.1 a	3.9 a	249.5 a	6.5 a	8.1 b	0.86 a	5.2 a

Table 4. Effect of drying after seed treatment on germination and growth characteristics of sorghum IRAT 204 under phytotron conditions.

Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time, GI: Germination Index, DWS: Dry Weight of Shoot, DWR: Dry Weight of Root, SRR: Shoot : Root Ratio and GUE: Germinated Unemerged Seeds.

### Effect of Salt Concentration, Osmoconditioning Treatment Duration and Storage

The germination pattern of treated seeds after storage revealed that in both the Wet Control and 8 g NaCl l<sup>-1</sup> treatment, a soaking duration of 2 days yielded better FGP values than 3 or 1 day soaking treatments, respectively. At the apparently high 16 g NaCl l<sup>-1</sup> level, soaking seeds for 1 day only was superior to soaking for 2 or 3 days (Table 5). Neither the MGT nor the GI were clearly affected by treatment and duration interactions. However, the highest GI value (best germination percentage and germination speed relationship) was observed in seeds treated with 8 g NaCl l<sup>-1</sup> for 2 days.

### Effect of Salt-Based Mixtures and Drying Duration

The interactive analysis of seed treatment, genotype and drying duration did not reveal significantly different germination percentages. From Table 6 it can be seen that the lowest FGP was observed in the NaCl + DAP fertilizer treatment. All seed osmoconditioning treatments reduced the MGT (seeds germinated faster) and increased the CVG (better germination percentage and rate) over untreated seeds. This means that seed osmoconditioning increased overall germination speed. Genotypes differed in their response to osmoconditioning with M35-1 performing in an in-

ferior manner in comparison to ICSV 112 and ICSV 745 as far as the FGP, MGT and CVG were concerned (Table 7). The duration of drying, on the other hand, did not affect any of the germination parameters studied (data not shown) pointing to the possibility of drying seeds after treatment in order to improve storage life.

Seed Treatment	Duration (days)	FGP (%)	MGT (day)	GI
Wet Control	1	78.6 bc	4.2 ab	255.0 b-d
	2	86.6 ab	5.2 a	219.3 cd
	3	72.6 c	4.7 ab	223.6 cd
8 g NaCl l <sup>-1</sup>	1	53.3 d	3.4 b	201.0 cd
	2	94.6 a	3.6 b	348.0 a
	3	86.6 ab	4.0 ab	298.3 ab
16 g NaCl l <sup>-1</sup>	1	84.0 bc	4.4 ab	275.3 bc
	2	58.0 d	4.1 ab	200.0 cd
	3	54.0 d	4.1 ab	185.3 d

Table 5. Interactive effects of NaCl concentration and soaking duration on the germination of sorghum IRAT 204 seeds after storage for 10 d under ambient conditions. Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time and GI: Germination Index.

Seed Treatment	FGP (%)	MGT (day)	CVG
Dry Control	74.2 b	3.7 a	27.7 c
NaCl (10 g/l) + Calcium Sulphate (5 g/l)	81.2 a	2.9 c	36.1 ab
NaCl (10 g/l) + Urea Fertilizer (7.5 g/l)	66.4 c	2.9 bc	33.9 ab
NaCl (10 g/l) + NPK Fertilizer (10 g/l)	71.0 bc	2.7 c	36.8 a
NaCl (10 g/l) + DAP Fertilizer (15 g/l)	56.8 d	3.1 b	32.7 b
NaCl (10 g/l)	68.7 bc	2.7 c	36.4 ab

Table 6. Effect of NaCl and NaCl-based seed osmoconditioning treatments on germination characteristics of sorghum varieties ICSV 112, ICSV 745 and M35-1 after storage for 1 month at 30°C. Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time and CVG: Coefficient of Velocity of Germination.

Genotype	FGP (%)	MGT (day)	CVG
ICSV 112	71.2 b	2.9 b	35.7 a
ICSV 745	84.4 a	2.9 b	34.9 a
M35-1	53.6 c	3.2 a	31.1 b

Table 7. Effect of genotype on germination characteristics of sorghum after storage for 1 month at 30°C. Means in columns followed by similar letters are not significantly different ( $\alpha \leq 0.05$ ). FGP: Final Germination Percentage, MGT: Mean Germination Time and CVG: Coefficient of Velocity of Germination.

From the results of the first experiment, which was carried out under simulated conditions in the phytotron, it is clear that NaCl concentration is decisive in the post-treatment germinative response seeds exhibit. While 8 g NaCl l<sup>-1</sup> improved germination, 16 g NaCl l<sup>-1</sup> retarded it in terms of FGP and GI. Both concentrations of salt, however, increased germination speed over water-soaked controls, but neither affected seedling growth or the fate of seeds which did not germinate. The longer the duration of soaking, the less positive effects were observed. Additionally, longer soaking durations (2 or 3 d in comparison to 1 d) almost doubled the number of germinated but unemerged seeds (Table 3). This response observed in fresh sown seeds was also observed in the second experiment where seeds were dried back and stored. The 8 g NaCl l<sup>-1</sup> and water-soaking treatments were better than 16 g NaCl l<sup>-1</sup> except that a 2-day treatment duration was observed to be better than 1 d for water and 8 g treatments, whereas 1 d was more suited for 16 g treatments. Again, 8 g NaCl l<sup>-1</sup> combined with a 2 d treatment duration advanced germination as seen from higher GI values. This shows that storage of treated seeds at ambient temperatures for a duration of 30 d does not negatively affect performance of pre-storage-hardened seed.

The fact that longer treatment durations increased the number of germinated unemerged seeds (first experiment) and reduced overall germination (16 g NaCl for 2 or 3 d in the second experiment) may be a direct effect of NaCl toxicity to seeds. That seeds germinate but do not emerge reflects a reduced vigor and/or abnormality (microscopic evidence, data not shown) since all seeds were planted at the same depth of 2 cm. Moisture supply was also regulated by weight and so the effect of external factors seems unlikely. A replication of the same treatments was conducted in a separate experiment and seeds/seedlings retrieved at 1, 3, 5 and 7 days after sowing. These were analysed for Na<sup>+</sup> and Cl<sup>-</sup> content and showed levels up to five times as high as non-treated seeds/seedlings. The results of this experiment will be reported in a

subsequent paper, but would indicate that ionic toxicity occurred. An analysis of hormonal levels would point to a sharp increase in ABA levels during soaking treatments and a mild increase during and after heat shock (Kader, unpublished data). This is where the hardening effect is likely to have taken effect, but at excessive NaCl levels a germination block would have occurred due to the shortage in Kinetin and GA<sub>3</sub> as a direct result of this physiological shock (Al-Mudaris 1998, Kader and Jutzi 2002). This points to the possibility that hardening can only provide alleviation from stress at moderate stress levels and becomes a stress itself at higher degrees of heat, drought or salinity (Noe and Zedler 2000).

The observation that 1 d was better for 16 g NaCl l<sup>-1</sup> treatments tends to confirm the toxicity hypothesis, since 1 d would not be enough to inflict such stress due to the uptake of water by the seed at high rates during the first day of treatment (Al-Mudaris and Jutzi 1998ab). Bussell and Gray (1976), in their work on osmoconditioning tomato seeds at -5 to -15 bars, observed that the length of soaking period had little effect at low osmotic potentials but not at high potentials. The 8 and 16 g NaCl l<sup>-1</sup> treatments measured -6.02 and -11.1 bar on the osmometer, respectively. This means that longer durations would expose the seeds to more osmotic stress.

Soaking sorghum in water alone, on the other hand, has been reported to increase germination speed as soaking period increased (Harris 1996). This has also been reported for pepper seed treated with the non toxic, inert manitol (Georghiou et al. 1987). The problem, then, lies within the nature of the osmoticum used. Seeds coming into contact with NaCl solutions have been found to have higher Cl<sup>-</sup> concentrations in their different components, the accumulation of which, in the embryo (Eschie 1995) and endosperm (Al-Mudaris, 1998), was associated with germination failure. Fulbright (1988) observed reduced germination in Indiangrass (*Sorghastrum nutans* cv. Cheyenne, Lometa) at 0.12 mol NaCl l<sup>-1</sup> (6.9 g NaCl l<sup>-1</sup>),

whereas Shanmugasundaram and Kannaiyan (1989) found 1.0% NaCl (10 g NaCl l<sup>-1</sup>) to give the highest germination percentage in pearl millet (*Pennisetum glaucum* L. R. Br.) in comparison to 2.5% (25 g NaCl l<sup>-1</sup>).

Increasing the salt concentration of a solution in contact with sorghum seeds reduces  $\alpha$ -amylase and protease activity, reducing and non-reducing sugar contents and the rate of reserve protein mobilization (Khan et al. 1989). Additionally, if a seed takes up solutes or mobilizes its reserves in an osmotically active form, its own water potential will be reduced although physiological processes may be inhibited both by the low water potential and the toxicity of ions (Bannister 1978). Ells (1963) reported that the priming effect of seed treatment with nutrient solutions (including 2% NaCl) is not due to the salts, nor to the amount of water retained by the seed from the treatment, but rather to certain enzymatic activities which take place within the seed while it is being held in a moist condition.

Reduced emergence, after seed germination (germination in the seedbed, but lack of emergence above the soil surface) was observed in the phytotron trial. If the radicle emerges from the testa and the soil water content is reduced below the initial soil water content due to drying, emergence is reduced (Helms et al. 1996). This could have been the case where trays were re-irrigated by weight only when they lost one third of their initial moisture content. This would have been more deleterious to seeds osmoconditioned for longer periods due to their higher initial moisture contents and would have led to a loss of the capacity to emerge (Peske 1983).

Combining NaCl with calcium sulphate improved seed response as seen from the third experiment, whereas other mixtures did not raise the final germination percentage but improved germination speed over controls. This may be due to the fact that the fertilizers used were, in themselves, salts and as such contributed to the ionic stress discussed above. A notable point is that of solubility. Calcium sulphate was

not totally soluble at the rates used, whereas sodium chloride was. The NPK fertilizer was also only partly soluble, whereas urea and DAP were more soluble.

Mixing sodium chloride with calcium probably increased Ca<sup>+2</sup> content of seeds (Bharati and Vaidehi 1989, Al-Mudaris 1998, Kader, unpublished data). Calcium has been found to play a major role in tolerance to NaCl where tolerant genotypes of vegetables contained higher Ca<sup>+2</sup> reserves in their seeds (Guerrier 1983). It has also been noted as modifying seed response to heat shock in maize (Gong et al. 1997) due, probably, to increasing membrane stability (Marschner 1995). Amzallag et al. (1997) also reported that leaf malformations in plants exposed to high NaCl concentrations were prevented by the addition of Ca<sup>+2</sup> to the nutrient solution.

Drying duration did not affect seed response to osmoconditioning. This agrees with the results of Emmerich and Hardegee (1996) who found that the germination of four warm-season grasses was not affected by length of the dehydration period. It is also in line with the work of Brocklehurst and Dearman (1984) who found that drying vegetable seeds after treatment did not interact with either the priming chemical used or the species tested and those of Al-Mudaris and Jutzi (1998ab) and Al-Mudaris (1998a). Drying the seed slowly by controlling humidity may impact germination rates achieved through conditioning (Mueller 1996). The loss of cell membrane integrity during drying is repaired when seeds are allowed to imbibe water, albeit after a certain period (Knypl and Khan 1981). It follows that the rate of drying (Dell Aquila and Tritto 1990) and its timing after initial imbibition (Kutschera 1995) play the major roles (Al-Mudaris and Jutzi 1999 and 1999a). Both the degree of drying and its timing used here seem to fall within the range which does not alter osmoconditioning effects. This ranged between 14 and 19.5% moisture content following treatment and after drying prior to storage.

## CONCLUSIONS

The three experiments were carried out under varying temperature and drought stress situations and after storage at ambient temperatures. The response of sorghum seeds to osmoconditioning in all three cases was generally more affected by the priming agent itself, its concentration and the duration of treatment than by drying or storage. This drying and storage is of great practical significance in the field, for if hardening is to be practiced, convenience in handling seeds must be fostered. This is difficult to achieve in moist batches of seed, which would be prey to fungal infection and render sowing a difficult task.

In conclusion, it would appear that seed hardening of sorghum via an NaCl-based soak has some potential to improve performance under post-treatment supra-optimal environmental conditions like drought or heat stress. The decisive factor in this is the thin line between this hardening actually inducing germination and it being an additional plant stress factor itself.

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- (Manuscript received 5.11.2002)  
 (Manuscript received in final form 04.02.2003)



## Edgeworth David Medal 2002

PROFESSOR MARCELA BILEK

Professor Marcela Bilek was appointed Professor of Applied Physics at Sydney University in 2000. She graduated from Sydney University with the University Medal in Physics in 1991 and has since worked in a number laboratories on projects that include atomic scale computer simulation, plasma processing, thin film materials and surface modification. In 1993 she was awarded the Minerals, Metals and Materials Society Reduction Technology Prize for her successful computer simulation of bubble stirring effects in aluminium cells, an achievement of considerable economic importance to the aluminium smelting industry.

A Peterhouse College and Cambridge Commonwealth Trust scholarship enabled her to obtain a PhD from Cambridge University in plasma technology for the fabrication of thin solid films. She continued similar work at Cambridge under an Emmanuel College Research Fellowship. Specialised materials, increasingly needed in microelectronics, biomaterials and optics, are enhanced by these methods. Much of this work has been done in collaboration with research groups in Australia.

She was the first to accurately model the transport of cathodic arc plasmas through mag-

netic filters, enabling the removal of microparticles from beams and produce beams with homogeneous cross sections for uniformly processing large wafers. Her models for the structure of both hydrogenated amorphous carbon and hydrogenated silicon carbide, based on quantum mechanical treatment of the bonding electrons, has been confirmed by numerous experimental observations. One socially significant example of her current research is the surface coating of materials to be implanted in the human body as prosthetic devices. Biocompatibility, adhesion and corrosion resistance is always a problem in the body which sees such devices as foreign and attempts to remove them. Her plasma implantation methods promise to significantly extend the useful life of such devices.

She has raised more than a million dollars for research since her Sydney appointment, convened a conference on biological effects of microwave radiation, delivered the 14th Pollock Lecture, been awarded a Young Tall Poppy Award 2001 by the Australian Institute of Political Science and the 2002 Malcolm McIntosh Prize for Physical Scientist of the Year.

JCK

## The Clarke Medal for 2002

PROFESSOR ROBERT HILL

Professor Robert Hill is a Senior Research Fellow in the School of Earth and Environmental Sciences at the University of Adelaide and is Head of Science at the South Australian Museum. He is a graduate of the University of Adelaide. He completed his Ph.D. on Tertiary plant macrofossils in 1981, and his D.Sc. on the interaction between climate change and the evolution of the living Australian vegetation in 1997. In 1979 he accepted a position as Tutor in Botany at James Cook University, and in 1980 was offered a lecturing position in the Department of Botany at the University of Tasmania where he remained until 1999, being promoted to Professor in 1993. He was Head of the School of Plant Science for 6 years prior to his departure, and was awarded Professor Emeritus status by the University of Tasmania Council in 2000. In 1999 he returned to the University of Adelaide to take up his current position.

Professor Hill has had a lifetime interest in the evolution of the vegetation in Australia and Antarctica. He has published more than 125 refereed journal papers, 35 book chapters, several symposium papers and has edited or co-

edited four books, including *The History of the Australian Vegetation* (Cambridge University Press), *Ecology of the Southern Conifers* (Melbourne University Press), *The Ecology and Biogeography of Nothofagus Forests* (Yale University Press), and *Vegetation of Tasmania* (Australian Biological Resources Study).

Professor Hill is President and a Fellow of the Australian Institute of Biology and a Fellow of the Linnean Society of London. His current research interest is the adaptation of the Australian vegetation to increasing aridity during the last 30 million years. He is developing a research program on the impact of fire on the Australian vegetation during this time period. He is best known for his research on the fossil history of the southern beech, *Nothofagus*, and the southern conifers. His work on the fossil history of *Nothofagus* has been critical in refining our understanding of its evolution and has led to a major revision of our understanding of the biogeography of this critical southern genus.

JCK

## Biographical Memoir



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**SIR ARTHUR RODEN CUTLER, V.C., AK,  
K.C.M.G., K.C.V.O., C.B.E., K. St.J.,**  
B.Ec (Syd.), LL.D (Hon.) (Syd.),  
D.Sc. (Hon.) (NSW & Newcastle)  
1916–2002

Arthur Roden Cutler, known as Sir Roden, was born on 24 May, 1916, at Manly, son of Arthur William Cutler and Ruby Daphne (née Pope) of Bathurst.

The Cutler family arrived in New South Wales as free settlers in 1833. The Roden family, of which his mother was a direct descendent, arrived even earlier (1827) with the army.

Roden Cutler was educated at Sydney Boys' High School and Sydney University, graduating B.Ec in 1935. At the University he excelled in sports, gaining Blues from both Sydney and the Australian Universities in swimming, and

participating in water polo and shooting. As a teenager he made an heroic surf rescue, risking his life against a large shark.

He joined the Public Trust Office of NSW on graduating, taking leave in 1940 to join the A.I.F. (2/5 Field Regiment). Roden Cutler's war service was relatively short, but spectacular, and became legendary. In Syria on 19 June, 1941 he showed "exceptional courage" in driving the enemy back and establishing outposts which were important factors in capturing Merdijayoun. Three weeks later (6 July), at Damour, he went forward against heavy machine-gun fire, was severely wounded, and subsequently had his leg amputated. He was awarded the Victoria Cross on 28 November, 1941.

Between 1942–43 he was a member of the Commonwealth Aliens Classification and Advisory Committee, Assistant Deputy Director of

the Security Service of NSW (1943), and from 1943 to 1946 Assistant Commissioner of the Repatriation Department. He was also NSW State Secretary for the R.S.L, 1942–43.

In 1946 he married Helen Gray Annetta Morris (d. 1990) and a new career path opened with his appointment as High Commissioner to New Zealand (to 1952), to Ceylon (now Sri Lanka), and the equivalent position (Australian Minister) to Egypt between 1955–56. Then followed two years in Canberra where he was Chief of Protocol in the Department of External Affairs, Secretary-General of a S.E.A.T.O. Meeting, and ACT President of the RSL.

In 1959 he moved overseas again as High Commissioner to Pakistan, with a brief interlude in the Somali Republic, and then to New York, where he was Australian Consul-General from 1961–65, and a delegate to the United Nations. His final overseas posting was as Ambassador to the Netherlands, 1965–66.

His association with this Society began in 1966, when he was appointed Governor of New South Wales, a post he held until 1981, the longest-serving Governor of the State. During this time he was also Administrator of Australia (acting Governor-General) on six occasions. He accepted the Council's invitation to joint Patronage (with the Governor-General), and was a strong supporter (with his wife) of the Society, during a period of turmoil, with the move from Science House and a considerable drain of the Society's finances.

At the Centenary Dinner in 1966 Sir Roden stressed the importance of scientific work

in modern times, and the contribution made by Australian scientists.

Sir Roden's speech at the 1974 Dinner, held at the Sydney Opera House, was memorable, marked as it was by some humorous comparisons with former Governors who had been associated with the Society (and its ancestors), but with discussion of more serious Society matters. He said "The Royal Society of New South Wales may not be as widely known as it deserves, nor may its functions be fully understood". While there was encouragement that membership had increased in the previous year, "the real value is in the learned qualifications of your membership, not in the total number", and the medals were held in high regard.

"The Society's task is to bring a balance into people's assessment of the advantages and limitations of scientific progress. You need to encourage research and investigation, and occasionally express a word of warning". He commended a "most valuable function" of the Society, the "keeping of a library," which he saw in its final open arrangement when opening the the Science Centre in 1977.

Sir Roden displayed in his speeches a good knowledge of the workings of the Society, despite the many other calls on his time, and his widespread interests. He died on 21 February, 2002 after a short illness, and is survived by his second wife, Lady Jane C. Cutler and four sons from his first marriage. The Society was represented at the State Funeral.

DFB

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JOURNAL AND PROCEEDINGS  
OF THE  
**R O Y A L   S O C I E T Y**  
**O   F**  
**N E W   S O U T H   W A L E S**

Volume 135 Parts 1 to 4  
(Nos 403 – 406)

**2003**

ISSN 0035-9173

PUBLISHED BY THE SOCIETY  
PO BOX 1525, MACQUARIE CENTRE, NSW 2113  
Issued June 2002 (Parts 1 and 2), April 2003 (Parts 3 and 4)

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ISSN 0035-9173

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ADDRESS      Royal Society of New South Wales,  
PO Box 1525, Macquarie Centre, NSW 2113, Australia  
<http://nsw.royalsoc.org.au>

DATE OF PUBLICATION      April 2003